EFFECT OF METHYLPREDNISOLONE ON THE LETHALITY OF ENDOTOXEMIC MICE

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13. ABSTRACT

In order to establish a reliable control for E. coli endotoxin studies, the lethality of various doses of endotoxin was tested in mice. Endotoxin (10 mg/kg, i.p. and 30 mg/kg, i.p.) resulted in a lethality of 60% and 87%, respectively. Methylprednisolone given 1 hr before endotoxin increased survival to 75% as compared to the 13% survival of the group which received endotoxin alone. Methylprednisolone (30 mg/kg, s.c.) increased survival markedly when given 1 hour before, simultaneous with, and from 1-6 hours after E. coli endotoxin (10 mg/kg, i.p.). Methylprednisolone has both a protective and a therapeutic effect on the survival of mice treated with E. coli endotoxin.

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INTRODUCTION

Generally, corticosteroids are considered as a good protection in shock states (Lefer and Verrier, 1970). In cases of septic shock caused by gram-negative organisms, many investigators have reported increased survival among the patients which were treated with massive doses of corticosteroids (Weil et al., 1964; Dietzman et al., 1967; Cavanaugh et al., 1968 and Motsay et al., 1972). These investigators also recommended highly the use of corticosteroids in the treatment of septic shock. Corticosteroids are considered beneficial because they exert several pharmacological actions including vasodilation, increased myocardial contractility, suppresion of inflammation and stabilization of lysosomal membranes. In spite of their common therapeutic use, no well-controlled studies in humans have been done to establish clearly their therapeutic efficacy in septic shock (Robson, 1971). The lack of good control also applies to dog endotoxin shock models. There, the uncertain biological status of the control animals and variability due to the usually small number of animals per study cause an unreliable condition for endotoxin shock survival, mechanism and therapy studies (Fine, 1966).

For toxicological studies, mice are obviously more advantageous than mongrel dogs since there are considerably less pharmacogenetic and physiological variations in mice. Furthermore, mice are less expensive so that relatively large numbers of animals can be used for more meaningful statistical analysis. Hence, the present study was undertaken to establish LD_{50} of E. coli endotoxin in male adult albino mice, and also to compare the effect of methylprednisolone on the lethality of endotoxin-treated mice to that of control mice.

METHODS

A total of 339 adult male albino mice were used in this study. All mice were kept in the local animal facility for a period of time ranging from 5-7 days to accustom them to the change in environment and diet before they became experimental subjects.

The first Section contained 129 mice which were divided into three groups. Each group received a different dose of E. coli endotoxin: Group A (10 mice) 10 mg/kg, i.p.; Group B (60 mice) 30 mg/kg, i.p. and Group C (59 mice) 10 mg/kg i.p. The mice were inspected hourly for 48 hours and the number of survivors was recorded. All mice alive at the end of 48 hours after endotoxin were considered to be survivors. The percentage which died in each group was taken as the measure of lethality of that particular dose of E. coli endotoxin in mice.

The second Section contained 132 mice which were divided into four groups. All groups received E. coli endotoxin (30 mg/kg i.p.). Group A (60 mice) is the same as Group B in the first section and it did not receive any other drugs. The remaining three groups received a different dose of methylprednisolone (Solu-Medrol) one hour before endotoxin: Group B (20 mice) 6 mg/kg, s.c.; Group C (20 mice) 30 mg/kg, s.c. and Group D (32 mice) 150 mg/kg,s.c. All mice were inspected hourly for 48 hours and the number of survivors was recorded. The percentage of survival was then calculated for each group and the values were compared.

The third Section contained 197 mice which were divided into three groups. Each group received E. coli endotoxin (10 mg/kg, i.p.). Group A (59 mice) is the same as Group C in the first section and it did not receive any other drugs. The remaining two groups received different doses of methylprednisolone

(Solu-Medrol) before, simultaneous with and after their injection of endotoxin: Group B (69 mice) 30 mg/kg, s.c. and Group C (69 mice) 150 mg/kg s.c. The methylprednisolone was administered to Groups B and C according to the following schedule:

1 hour before endotoxin (12 mice each group); simultaneous with endotoxin (12 mice each group); 1 hour after endotoxin (12 mice each group); 2 hours after endotoxin (12 mice each group; 4 hours after endotoxin (11 mice each group) and 6 hours after endotoxin (10 mice each group). The mice were inspected hourly for 48 hours and the number of survivors was recorded. The percentage of survivial was computed for each group and the values were compared.

Materials

Mice were obtained from Arthur Sutter, Springfield, Missouri and The Mouse House, Marlow, Oklahoma 73055. Lipopolysaccharide B. E. Coli 0127; B8 (batch 209684) was obtained from Difco Laboratories, Detroit, Michigan.

RESULTS

The lethality of various doses of E. coli endotoxin in mice is shown in Fig. 1. After 48 hours, endotoxin (10 mg/kg, i.p.) exhibits a 60% lethality (LD_{60}). The higher doses of endotoxin (30 mg/kg, i.p. and 100 mg/kg, i.p) are highly lethal in mice, 87% and 90%, respectively.

As shown in Fig. 2., mice which received methylprednisolone 1 hour before E. coli endotoxin (30 mg/kg, i.p.) survived in greater numbers than those which received only endotoxin. 75% of the mice in the methylprednisolone (30 mg/kg, s.c.) group survived as compared to 13.3% of the group which received endotoxin alone. The other methylprednisolone (6 mg/kg, s.c. and 150 mg/kg, s.c.) groups also had a high percentage of survival, 29.5% and 56.2%, respectively.

Methylprednisolone was also shown (Fig. 3) to enhance survival of mice when administered simultaneously with and after E. coli endotoxin (10 mg/kg, i.p.). The survival of mice which received either dose of methylprednisolone 1 hour before endotoxin was increased compared to the group which received endotoxin alone. This effect was also shown in Fig. 2. All mice receiving methylprednisolone with endotoxin (0 TIME) also exhibited an enhanced survival, especially the group receiving methylprednisolone (150 mg/kg, s.c.), in which all animals survived. In all groups administered methylprednisolone (30 mg/kg, s.c.) 1 to 6 hours after endotoxin, the survival percentage was increased 10%-40% above that for the groups which received endotoxin alone (40%). At 1, 2 and 4 hours after endotoxin, the administration of methylprednisolone (30 mg/kg, s.c.) greatly increased survival. There was little effect, however, on survival by the higher dose of methylprednisolone (150 mg/kg, s.c.) when administered after endotoxin, except at 2 hours, when it decreased the percentage of survival below that of the group given endotoxin alone.

DISCUSSION

A consistent percentage of lethality was found among a large number of mice when E. coli endotoxin was administered (Fig. 1). Endotoxin (10 mg/kg, i.p.) produced a 60% lethality (LD_{60}) in which most animals died between 6 and 24 hours after endotoxin administration. This dose of endotoxin in mice will provide a useful control for studying the protective and therapeutic value of drugs such as methylprednisolone (Fig. 3). Further, it will provide a good model for studying the mechanism of irreversible endotoxin shock as well as the reversal of such shock states. As shown in Fig. 3., methylprednisolone (30 mg/kg, s.c.) obviously reverses the lethality of E. coli endotoxin in mice when it is given after endotoxin. Endotoxin (30 mg/kg, i.p.) produces a greater lethality in mice (87%), which may be too severe for consistent therapeutic studies. However, pretreatment with methylprednisolone (30 mg/kg, s.c. and 150 mg/kg, s.c.) resulted in dramatic protection from the lethal effects of this dose of endotoxin (Fig. 2). Endotoxin (100 mg/kg, i.p.) appears to be too large a dose to be useful in therapeutic studies.

Methylprednisolone has a marked protective effect on mice when given 1 hour before E. coli endotoxin and simultaneous with E. coli endotoxin (Fig. 2 and 3). After endotoxin, the smaller dose of methylprednisolone (30 mg/kg, s.c.) had a therapeutic effect. Weil (1962) also showed a therapeutic value of corticosteroids in mice given endotoxin. The larger dose of methylprednisolone (150 mg/kg,s.c.), however, had no effect on survival when given 1 and 4 hours after endotoxin, and it actually decreased survival when given 2 hours after endotoxin. The reason for this effect is unclear, but it seems to be related to the stage of shock during which the drug is administered.

The protective effect of methylprednisolone has also been seen in dogs by Thomas and Brockman (1968), but they did not find a therapeutic effect throughout extensive studies. Hinshaw et al. (1967), however, demonstrated a marked therapeutic effect of methylprednisolone administered in dogs following E. coli endotoxin. Fine (1966) reported the unreliability of endotoxin-treated control dogs. The survival of 9 endotoxin-treated groups containing a total of 38 dogs in a study in which several groups of investigators were involved ranged from 0% to 50%. Despite careful control studies, it is difficult to assess LD_{50} or LD_{80} doses due to variability of individual dogs and of different breeds of dogs. In order to do extensive studies in a problem, it is necessary to have animals which exhibit a consistent response to the same condition. Mice appear to be more consistent in their response to endotoxin than dogs are and they may therefor become a valuable experimental model for E. coli endotoxin shock.

SUMMARY

In order to establish a reliable control for E. coli endotoxin studies, the lethality of various doses of endotoxin was tested in mice. Endotoxin (10 mg/kg, i.p. and 30 mg/kg, i.p.) resulted in a lethality of 60% and 87%, respectively. Methylprednisolone give 1 hour before endotoxin increased survival to 75% as compared to the 13% survival of the group which received endotoxin alone. Methylprednisolone (30 mg/kg, s.c.) increased survival markedly when given 1 hour before, simultaneous with, and from 1-6 hours after E. coli endotoxin (10 mg/kg, i.p.). Methylprednisolone has both a protective and a therapeutic effect on the survival of mice treated with E. coli endotoxin.

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LETHALITY IN MICE PRODUCED BY THE ADMINISTRATION OF E. COLI ENDOTOXIN IN VARYING DOSES

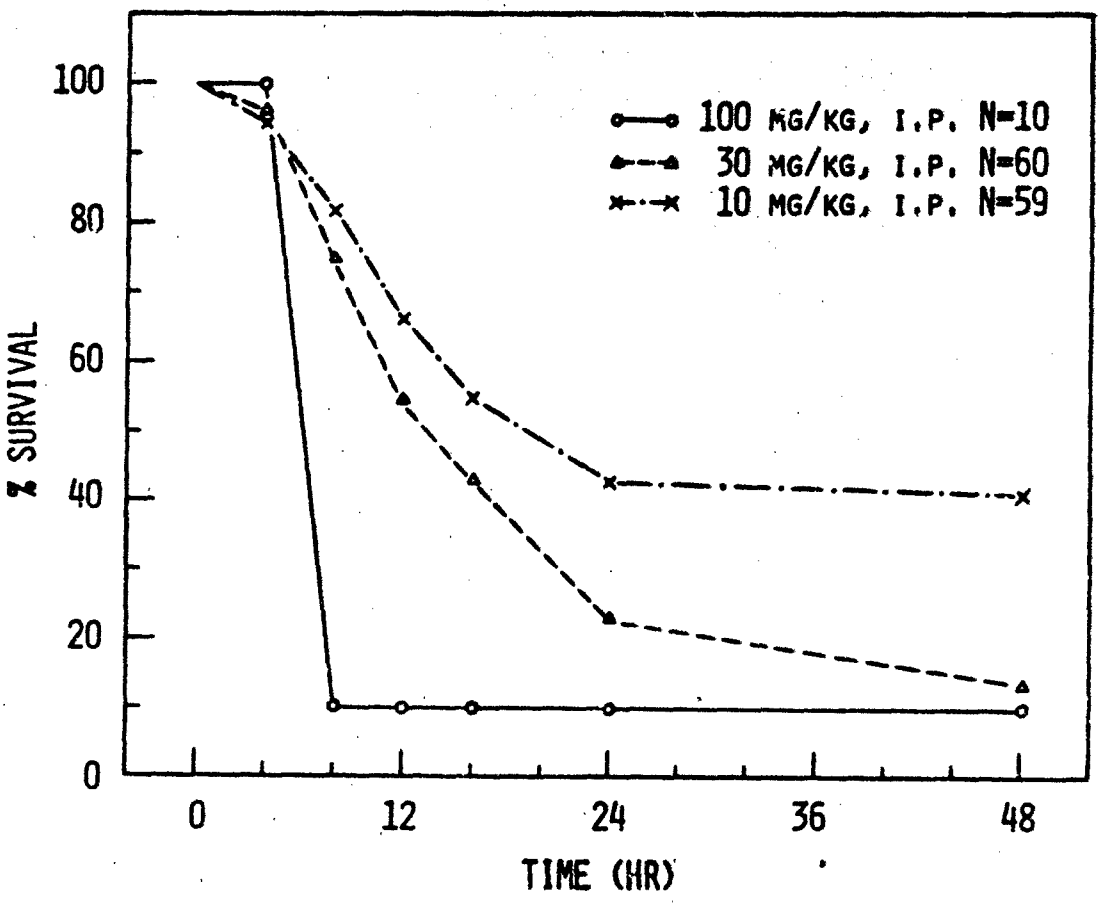

FIGURE 1

THE EFFECT OF METHYLPREDNISOLONE (MP) ON THE SURVIVAL OF MICE GIVEN E. COLI ENDOTOXIN (30 MG/KG, I.P.)

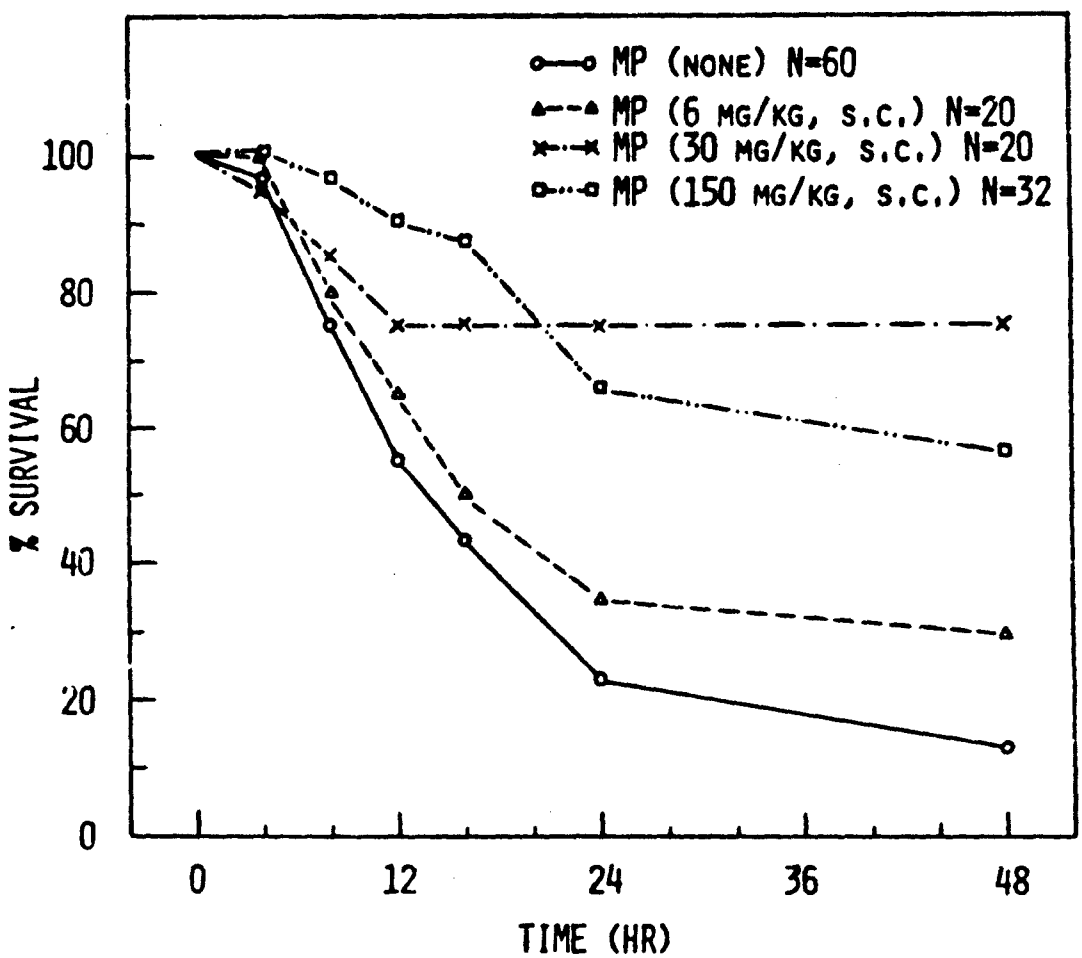

FIGURE 2

THE EFFECT OF METHYLPREDNISOLONE (MP) ON THE SURVIVAL OF MICE WHEN GIVEN BEFORE, SIMULTANEOUS WITH, AND AFTER E. COLI ENDOTOXIN (10 MG/KG, I.P.)

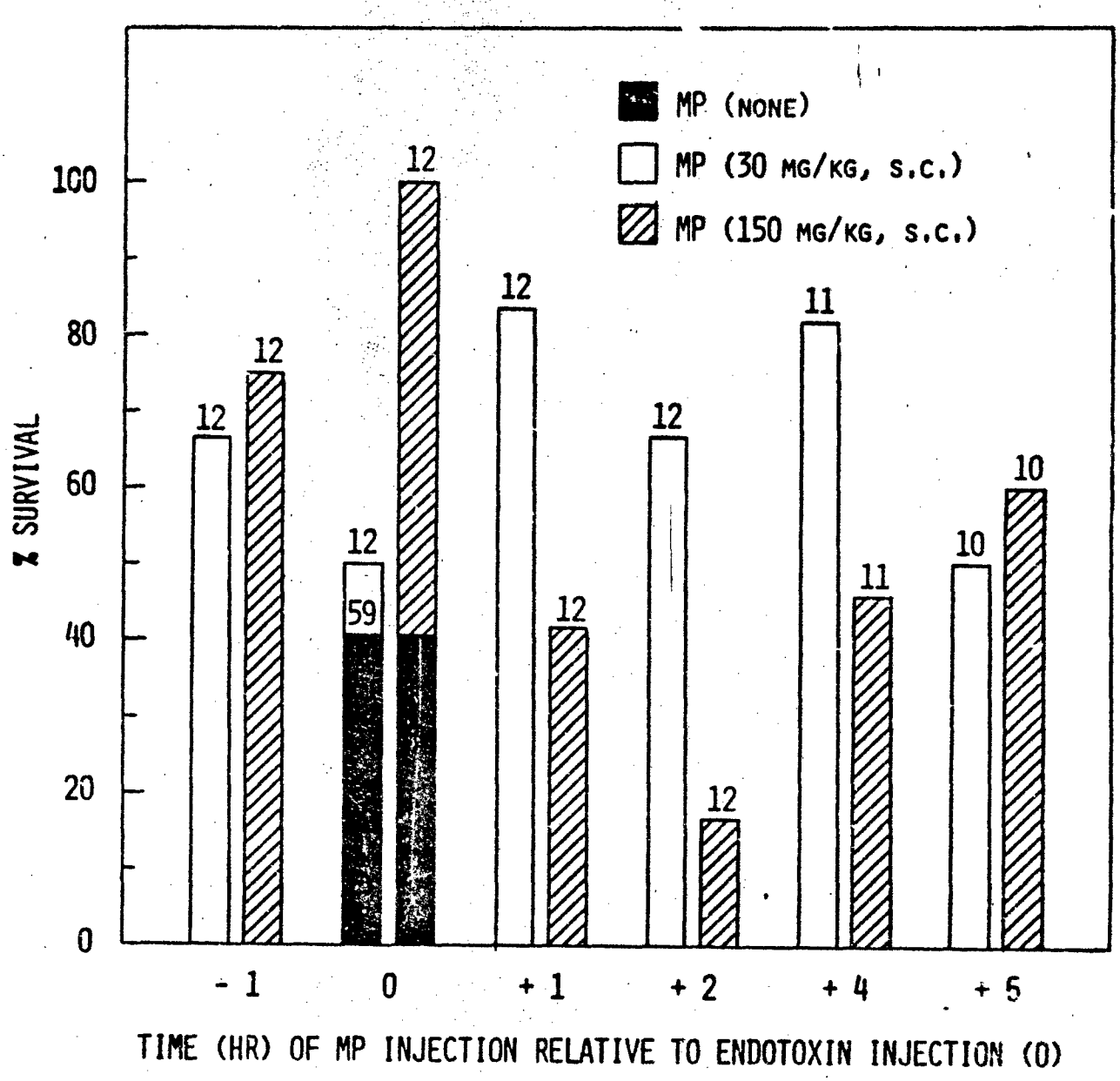

FIGURE 3